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PATENT Attorney Docket No. DHI-06207

Examiner: Shanon A. Foley

Group No.: 1648

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Yung T. Huang

Filed:

Entitled:

Serial No.:

09/844,311

04/27/01

CELLS FOR DETECTION OF

ENTEROVIRUSES

DECLARATION UNDER 1.132 BY DR. YUNG T. HUANG

Mail Stop - AF Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. § 1.8(a)(1)(7)(B)

I hereby certify that this correspondence (along with any documents referred to as being attached or enclosed) is, on the date shown below, being transmitted by facsimile to the Patent and Trademerk Office.

Madam:

- I, Yung T. Huang, am inventor of the pending claims in the instant application, and am the subject of the attached Curriculum Vitae (Tab 1) and author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents, I submit that I am qualified to speak on the level of ordinary skill in the art of the claimed invention.
- 2. The Examiner rejected Claims 1-4 and 6-14 as being allegedly obvious over Scholl et al. and Powell et al.,23 and also rejected Claim 5 under 35 U.S.C. §103(a) for

US patent 6,168,915.

Powell et al. (1998) J. Gen. Virol. 79:1707-1713.

Paper No. 17, middle of page 3.

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alleged obviousness over Scholl et al., Powell et al., Spiller et al., and either the sequence alignment of SEQ ID NO:1 with GenEmbl accession no. M15799 of Medoff et al., or the sequence alignment of SEQ ID NO:3 with GenEmbl accession M30142 of Caras et al., or the

3. It is my opinion that the cited references neither provide motivation to make the claimed compositions, nor provide a reasonable expectation of success in making and using the recited compositions for the reasons explained below.

i. The references do not provide motivation to make the claimed compositions

We previously argued that Powell et al. failed to confer permissiveness to CHO and RD cell transfected with DAF and concluded that enteroviruses "can indeed use alternative cellular receptors" different from DAF. Since it is unknown whether enteroviruses use DAF or another unknown receptor for binding to BGMK cells, then there is no motivation to transfect BGMK with DAF (as opposed to transfection with some other unknown receptor).

The Examiner responded that "While Powell et al. increase the susceptibility in mouse cells, increased permissiveness to enterovirus infection in a cell expressing a recombinant DAF receptor would only increase susceptibility in a cell that is already susceptible to enterovirus infection, i.e. buffalo green monkey cells. This is due to the established fact in the art that Buffalo green monkey cells are already susceptible to enterovirus infection."

However, The Examiner's assumption is factually wrong because, my experiments that are described in the Specification show that DAF did not alter either sensitivity to enterovirus, or permissiveness to Echo-6 or -11 when DAF was used to transfect H292 cells, even though H292 cells are "already susceptible to enterovirus infection."

For example, the Specification states that:

Spiller et al. (2000) J. Infectious Diseases 181:340-343.

Medoff et al. (1987) PNAS 84(7):2007-2011.

Caras et al. (1987) Nature 325-(6104):545-549.

Paper No. 17, paragraph bridging pages 6 and 7.

⁽Emphasis added) Paper No. 17, page 4, bottom third.

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"The properties and advantages of the invention's transgenic BGMk cells were surprising in view of contrary data disclosed herein when using transgenic H292 cells. In particular, data disclosed herein demonstrates that, whereas transfection of BGMK cells with vectors that express human decay accelerating factor increased both the sensitivity and permissiveness of BGMK cells to enteroviruses, in contrast, no increase in sensitivity to enteroviruses was observed when the same vectors were used to transfect H292 cells."

My results that are disclosed in the Specification using both laboratory isolates¹⁰ and clinical samples¹¹ demonstrate:

"...that transfection of additional copies of the hDAF gene into the H292 cells which express hDAF did not increase or decrease the cells' sensitivity for the detection of laboratory strains of enteroviruses. These results are in direct contrast to those obtained with BGMK-hDAF cells shown in Example 2 supra." "In other words, transfection of additional copies of the hDAF gene into H292 cells had no effect on the sensitivity of detection of enteroviruses from in clinical specimens by these cells."

Thus, my results were "surprising" in the face of Scholl et al.'s and Powell et al.'s disclosure.

Importantly, also, nothing in the art teaches that DAF will confer a new property on the cells i.e., the recited property of selective permissiveness to echovirus-6 and/or echovirus-11 (claim 2 and 4). Nothing in the references suggests that wild type BGMK cells, which are not susceptible to either echovirus-6 or -11, would become susceptible to these particular strains when BGMK cells are trasfected to express DAF.¹⁴

^{9 (}Emphasis added) Specification, page 6, lines 11-17.

Example 5 beginning on page 50 of the Specification.

Example 6 beginning on page 54 of the Specification.

⁽Emphasis added) Specification, page 54, liens 6-9.

⁽Emphasis added) Specification, page 55, lines 1-4.

The Specification confirms that "For echovirus-6 and echovirus-11, BGMK cells failed to detect these two viruses. Importantly, in contrast, BGMK-hDAF cells detected highly diluted virus by day 1."

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ii. A reasonable expectation of success in making and using the recited compositions is not established

We previously stated that the specification's data shows that transfecting H292 cells with DAF 'had no effect on the sensitivity of detection of enteroviruses..." Therefore, it is unpredictable whether increasing the number of copies of DAF in a cell (e.g., BGMK) that is already susceptible to enterovirus would increase sensitivity to the enterovirus.

The Examiner responded that "These results are not clearly unexpected due to the fact that it is established in the art that human tissue culture cells, i.e. H292, are not as susceptible to enterovirus infection compared with buffalo green monkey cells." In support, the Examiner stated that Melnick "clearly shows that human cell lines are not as susceptible to enterovirus infection compared to monkey kidney cell culture, see Table 2."

However, the Examiner's position is contradicted for at least the following six reasons. First, Melnick does not support the Examiner's position that "H292, are not as susceptible to enterovirus infection compared with buffalo green monkey cells" because Melnick does not make a direct comparison between the specific cell types of H292 (that we used in our investigation) and the invention's BGMK cells. Rather, Melnick's makes a generalized statement by comparing the cytopathic effect of enteroviruses in "monkey kidney" and "human" tissue cultures (Table 2 of Melnick).

Second, Melnick contradicts the Examiner's position by teaching away from using BGMK cells in favor of using human and primary monkey cells for echovirus detection because it says that BGMK cells are not as sensitive to echoviruses as human cells. For example, Melnick says that "...the [BGMK] line may have limitations in sensitivity for routine isolation of a variety of echovirus types, as compared with primary rhesus monkey kidney and human fetal diploid kidney cells."

Third, the Specification's data contradicts the Examiner's position that "H292, are not as susceptible to enterovirus infection compared with buffalo green monkey cells" by showing that transgenic H292 cells had the same or better sensitivity as transgenic BGMK cells when infected with Echovirus-4 (days 1-3) and Echovirus-9 (days 2-3) (Table 2 on page 41, and

⁽Emphasis added) Paper No. 17, page 6, second full paragraph.

Melnick et al., page 661, column 1, second full paragarph.

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Table 6 on page 51), as well as with Coxsackievirus B1 (days 1 and 3; Table 3 page 44 and Table 7 on page 53).

Fourth, Hierholzer et al. contradicts the Examiner's statement that "H292, are not as susceptible to enterovirus infection compared with buffalo green monkey cells," because Hierholzer shows that H292 cells supported enterovirus replication to the same extent as other preferred cell cultures. Hierholzer et al. observed "that (i) viruses replicated at about the same rate in H292 cells as in their usually preferred cell cultures ..., (ii) virus titer in H292 cells were comparable to those obtained in other, more traditionally preferred cells, and (iii) antigen titers ... were also comparable to those found in supernatant fluids from other cell lines."

Fifth, Dagan & Menegus contradicts the Examiner's statement that "H292, are not as susceptible to enterovirus infection compared with buffalo green monkey cells," because this reference (which is co-authored by Dr. Menegus, who is authoritative on enteroviruses) shows that BGMK cells are not as susceptible as human cell lines RD and HEL to enteroviruses. 18

Sixth, the Examiner ignored Applicant's remarks that Powell does not provide a reasonable expectation of success, since Powell failed to confer permissiveness and/or increase sensitivity to enterovirus when transecting RD and CHO cells with DAF. Powell's failure is relevant because it shows that DAF is sufficient to confer permissiveness to enteroviruses in some cases but not others, and there is no basis or guidance in any of the cited references for predicting when success is to be expected.

4. Based on my expertise in the relevant art, and in view of the above discussion, it is my opinion that one of ordinary skill in the art would not have been motivated to make the claimed compositions, nor provided with a reasonable expectation of success in making and using the recited compositions.

Hierholzer et al. page 1509, second column, second paragraph; and Table 1, page 1507.

Dagan & Menegus, Figure 1, on page 223.

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5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: March 158 2004

Dr. Yung T. Huang

TAB 1

Revised:Mar..03

CURRICULUM VITAE

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NATIONALITY:

U.S.A.

EDUCATION:

BS Degree

Tunghai University, Department of Biology, Taichung,

(1963)Taiwan.

MS Degree (1978)

University of North Carolina, Department of Bacteriology

and Immunology, Chapel Hill, N.C. 27514

PhD Degree

(1983)

University of North Carolina, Department of Microbiology

and Immunology, Chapel Hill, N.C. 27514

Postdoctoral

Dept. of Microbiology and Immunology, University of

North Carolina, Chapel Hill, N.C. 27514 (1983)

PROFESSIONAL APPOINTMENTS:

1984 - present:

Assistant to Associate Professor, Department of Pathology, Case Western Reserve University and Director of Clinical Virology/Serology, Department of Pathology, University Hospitals of Cleveland, Cleveland, Ohio 44106

1979-Jan. 1980:

Research analyst and supervisor, Tissue Culture Facility, Cancer Research

Center, University of North Carolina, Chapel Hill, N.C. 27514

1972-1978:

Supervisor, Virology Laboratory, Clinical Microbiology Laboratory, Department of Hospital Laboratories, North Carolina Memorial Hospital,

Chapel Hill, N.C. 27514

1970-1971:

Medical laboratory supervisor, U.S. Naval Medical Research Unit No. 2,

Taipei, Taiwan

1969-1970:

Special trainee and research technician. School of Public Health,

Casa a di Santa da Maria da M

University of Washington, Seattle, Washington

1964-1969:

Senior medical laboratory technician. U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan

MILITARY SERVICE:

1963-1964: Medical officer in Army (Taiwan).

PROFESSIONAL SOCIETIES:

Member, American Society of Microbiology Member, American Society of Virology

Member, Pan American Society for Clinical Virology

Member, American Association for the Advancement of Science

Member, American Society of Pathology

RESEARCH SUPPORT:

Biomedical Support Grant 1985-1986 (\$9,883)

Cystic Fibrosis Foundation, Rainbow Chapter 1987-1988 (\$13,546)

National Institute of Health, SCOR: Chronic Diseases of the Airways, 12/1/91 - 11/30/96, Principal Investigator, E. R. McFadden

Project Title: Interactions of neutrophils with airway epithelial cells in vitro, T.D.C. \$569,000 Principal Investigator, Pamela B. Davis, M.D. Co-investigator with 10% effort

Project Title: Stimulation and evaluation of mucosal immunity to respiratory pathogens, T.D.C. \$929,734

Principal Investigator, John G. Nedrud, Ph.D.

Co-principal investigator, Michael E. Lamm, M.D.

Co-investigator with 15% effort

National Institute of Health, Program project: Mucosal Immunity and Infection, 9/1/95 -8/30/98,

Project Director, M. E. Lamm

Principle Investigator with 30% effort

Project Title: Intracellular neutralization of SIV/HIV, T.D.C. \$144,000

Grant from Diagnostic Hybrids INC. Athens, Ohio. Project Title: Investigation of Turbo for viral diagnosis

1/1/97 - 12/31/98 T.D.C. \$40,000

National Institute of Health, Project: Studies on secretory Immunoglobulin 01-01-98 to 12-31-02

Principal Investigator: Michael E. Lamm

Co-investigator with 15% effort.

National Institute of Health, Project: FAS-Ligand Mediated Immunity to HSV.

07-01-99 to 06-30-2004

Principle Investigator: David Kaplan

Co-investigator with 4% effort

National Institute of Health,

Program project: Mucosal immunity and infection 10-1-00 to 9-30-05

Project 3: Principal Investigator

Project title: IgA in resisting HIV. TDC \$754, 179.00

Ohio Dept. of Development (Technology Action Fund).

Fund period: 6-03 to 5-05 Principle investigator

Project title: Genetically Engineered cell lines for Improving Influenza Vaccine Production and

Rapid Respiratory virus Detection.

Total funding: \$401,390.00

BOARD CERTIFICATION:

American Board of Bioanalysis: Certified High-Complexity Clinical Laboratory Director

OTHER:

First patent granted on April 6, 1999.
Second patent granted on January 2, 2001.
Third patent granted on August 28, 2001.
Fourth patent granted on October 23, 2001
Fifth patent granted on April 23, 2002
Sixth patent granted on April 25, 2002
Seventh patent granted on June 18, 2002
Eighth patent granted on December 17, 2002
Ninth patent granted on August 26, 2003
All licensed to Diagnostic Hybrids Inc. Athens, Ohio.

COMMITTEE APPOINTMENTS:

Hospital Laboratory Quality Improvement Committee, 1996 - 1998
Pediatric Infection Control Committee, 1990 - Present
Pathology Department Library Committee, 1995-Present

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PUBLICATIONS:

ORIGINAL PAPERS:

- 1. Lee GCY, Funk GA, Chen ST, <u>Huang YT</u>, and Wei HY. An outbreak of respiratory syncytial virus infection in an infant nursery. J Formosan Med Assoc 72:39-46, 1973
- 2. Huang FS, and Pagano JS. Specific antisera to cytomegaloviruses. J Immunol 112:528, 1974
- 3. Lee GCY, Huang YT, and Chang LC. Preliminary study of attenuated Japanese Encephalitis Virus. J Formosan Med Assoc 74:606-12, 1974
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- 8. Lemon SM, Hutt LM, <u>Huang YT</u>, Blum JE and Pagano JS. Simultaneous infection with multiple Herpes viruses. Amer J Med 66:270-76, 1979
- 9. Huang YT and Wertz G. Respiratory syncytial virus is a negative stranded RNA virus that code for at least seven complementary message RNA species. J Virol 43:150-57, 1982
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- Collins PL, Huang YT and Wertz GW. Identification of a tenth mRNA or respiratory syncytial virus and assignment of polypeptides to the ten viral genes. J Virol 49:572-78, 1984
- Collins PL, Huang YT and Wertz GW. Nucleotide sequence of the gene encoding the fusion (F) glycoprotein of human respiratory syncytial virus. Proc Natl Acad Sci 81:7683-87, 1984
- 13. Huang YT. Collins PL and Wertz GW. Characterization of the 10 proteins of human respiratory syncytial virus: Identification of a fourth envelope associated protein. Virus

Research 2:157-73, 1985

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- Midulla F, Huang YT, Gilbert IA, Cirino NM, McFadden ER, Jr and Panuska JR. Respiratory syncytial virus infection of human cord and adult blood monocytes and alveolar macrophages. Am Rev Respir Dis 140:771-77, 1989
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 Productive infection of isolated human alveolar macrophages by respiratory syncytial virus. J Clin Invest 86:113-19, 1990
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- 21. Stark JM, Fatemi SH, Amini SB, Huang YT. Occurrence of respiratory syncytial virus subtypes in hospitalized children in Cleveland, Ohio from 1985-1988. Pediatric Pulmonology 11:98-102, 1991
- 22. Stark JM, Huang YT. Davis PB. Infection of cultured human tracheal epithelial cells by human parainfluenza virus types 2 & 3. J Virological Methods 3:31-46, 1991
- 23. Tosi MF, Stark JM, Hamedani A, Smith CW, Gruenert DC, Huang YT. Intracellular adhesion Molecule I (ICAM-1)-dependent and ICAM-1-independent adhesive interactions between polymorpho-nuclear leukocytes and human airway epithelial cells infected with parainfluenza virus type 2. J Immunol 149:3345-49, 1992
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hybridization. J Virol Method 41:1-8, 1993

- Huang YT, Romito RR, Bishna PD, Banerjee AK. Characterization of the in vitro system for the synthesis of mRNA from human respiratory syncytis/virus. Virology 193:862-67, 1993
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- 27. Sieg S, Muno-Cacho C, Robertson S, <u>Huang YT</u>, Kaplan D. Immunoregulation of human T lymphocytes by parainfluenza virus type 3. Proc Natl Acad Sci USA, 91:6293-97, 1994
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- 36. Sieg S, King C, <u>Huang YT</u>, Kaplan D. The role of interleukin-10 in the inhibition of T-cell proliferation and apoptosis mediated by parainfluenza virus type 3. J Virol 70:4845-48, 1996

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- Huang YT. Turchek B. Mink lung cells and mixed mink lung and A549 cells for rapid detection of influenza virus and other respiratory viruses. J. Clinical Microbiol. 38: 422-423, 2000
- Huang YT, Hite S, Duane V, Yam P, and Jollick JA, Application of mixed cell lines for the detection of viruses from clinical specimens. Clinical Microbiology Newsletter. 22: 89-92, 2000
- Huang YT Hite S, Duane V, and Yan H. CV-1 and MRC-5 mixed cells for simultaneous detection of heroes simplex viruses and varicella zoster virus in skin lesions. J Clinical Virol. 24: 37-43, 2002
- Huang YT, Yam P, Yan H, and Sun Y. Engineering BGMK cells for sensitive and rapid detection of enteroviruses. J Clinical Microbiol. 40: 366-371, 2002
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1. Huang YT. Davis NL and Westz GW. Separation and characterization of the RNAs of human respiratory syncytial virus. IN: Replication of Negative Stranded RNA Viruses. Bishop, D.H.L. and Company, R.W., Eds. Elsevier, p. 531-36, 1981

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- 5. Huang YT Collins PL and Wertz GW. The polypeptides of human respiratory syncytial virus. Abstracts American Soc. Microbiology T-27, 1984
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- 7. Heggie AD, Roessmann U and <u>Huang YT</u> Host factors in coxsackie virus B-2 disease. Presented to annual meeting of American Pediatric Society, Washington D.C., May 1988
- 8. Weaver MG, Abdul-Karim FW, Dale G, Sorensen K, and Huang YT. Detection and localization of human papillomavirus in penile condylomas and squamous cell carcinomas using in situ hybridization with biotinylated DNA viral probes. Presented to United States and Canadian Academy of Pathology meeting, September 1988
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- 10. Stark JM, Huang YT, and Davis PB. Parainfluenza and respiratory syncytial virus infection of cultured human epithelial cells. A possible model for lower respiratory tract disease. Presented to the annual meeting of American Thoracic Society, November 1988
- 11. Midulla F, Huang YT, Gilbert I, Cirino N, McFadden ER and Panuska J. Respiratory syncytial virus infection of human mononuclear phagocytes. Presented to annual meeting of American Thoracic Society, January 1989
- 12. Tosi M, Stark JM, Hamedani A, Smith CW, Gruenert DC and <u>Huang YT</u>. Increased adhesion by neutrophils to human tracheal epithelial cells infected with parainfluenza virus type 2: role of epithelial ICAM-1 and neutrophil CD11/CD18 adhesions.

Presented to annual meeting of the American Pediatric Society, April 1991

- Sokhandan M, McFadden ER, Huang YT, Mazanec MB. The contribution of respiratory viruses to exacerbations of asthma in adults. Presented to annual meeting of the American Thoracic Society, May 1991
- 14. Heggie AD, Scholl DR and <u>Huang YT</u>. Rapid detection of herpes simplex viruses in culture by in situ hybridization. Presented to Annual Clinical Virology Symposium, April 1992
- Huang YT. Romito RR, De BP, Banerjee AK. In vitro RNA synthesis of human respiratory syncytial virus. Submitted to annual meeting of American Society of Virology, July 1992
- Panin M, Liang XP, Nedrud JG, <u>Huang YT</u>. Immune response in mice upon oral immunization with attenuated salmonella typhimurium expressing Np of Sendai virus. Submitted to annual meeting of the American Society of Virology, July 1992
- 17. Galinski MS, Hemingway BR, Yang Y, Panin M, <u>Huang YT</u>. Role of basic residues in the proteolytic activation of Sendai virus fusion glycoprotein. Submitted to the annual meeting of American Society of Virology, July 1993
- 18. Pappin A, Grissom M, <u>Huang YT</u>, Yomtovian R. Beyond the seven day limit: Stability of cytomegalovirus antibodies in plasma during prolonged red blood cell storage. Submitted to annual meeting of American Society of Clinical Pathology, August 1994
- 19. Hite S, <u>Huang YT</u>. Microwave-accelerated direct immunofluorescent staining for RSV and influenza virus. Submitted to the annual meeting of the North American Society for Clinical Virology, April 1995
- 20. Huang YT, Miller C, Nedrud J, Lamm M. Replication and budding of SIV in polarized epithelial cells. Submitted to the annual Symposium on Nonhuman Primate Models for AIDS, November 1995
- Huang YT, Dul J, Brown J, Scholl D. Shell vial detection of CMV using mink lung cells enhanced by treatment with CMV Turbo Treat™: A new viral culture medium. Submitted to the Annual Meeting of the North American Society for Clinical Virology, April 1997
- 22. Turchek B, Huang YT. Typing of HSV isolates detected by Elvis HSV test. Submitted to the Annual Meeting of the North American Society for Clinical Virology, April 1997
- 23. Friedland RP, Lerner AJ, Smith AL, <u>Huang YT</u>, Siedlak SL, Perry G. Latent infection with cytomegalovirus in Alzheimer's Disease (AD): Serum antibody and immunocytochemical studies. Submitted to the American Society for Neurology, 1997

- 24. Huang YT, Turchek B. Sensitive cell lines for detection of respiratory viruses from clinical specimens: comparison of mink lung and NCIH292 to primary rhesus monkey kidney and Hep-2 cells. Presented at Annual Meeting of the North American Society for Clinical Virology, April 1998
- Huang YT, Hite S, Duane V. CV-1/MRC-5 mixed cells for detection of herpes simplex viruses: comparison with primary rabbit kidney and mink lung cells. Presented at Annual Meeting of the North American Society for Clinical Virology, May 1999
- Huang YT, Evaluation of cryopreserved cell monolayers for the rapid detection of influenza A and herpes simplex viruses. Presented at Annual Meeting of the North American Society for Clinical Virology, April 2000
- Huang YT, Yam P, Jollick JA. CaCO2 and engineered BGMK mixed cells for the detection of enteroviruses. Presented at Annual Meeting of the North American Society for Clinical Virology, April 2001
- Yan H, Lamm ME, <u>Hunag YT</u>. Functions of IgA in defense of mucosal epithelium-an in vitro measles virus model. 11th International Congress of Mucosal Immunology, June 2002